

What is claimed is:

1. An isolated DNA encoding the enzyme I-SceI, wherein the DNA has the nucleotide sequence:

ATG CAT ATG AAA AAC ATC AAA AAA AAC CAG GTA ATG 2670
M H M K N I K K N Q V M 12

2671 AAC CTC GGT CCG AAC TCT AAA CTG CTG AAA GAA TAC AAA TCC CAG CTG ATC GAA CTG AAC 2730
13 N L G P N S K L L R E Y K S Q L I E L N 32

2731 ATC GAA CAG TTC GAA GCA GGT ATC GGT CTG ATC CTG GGT GAT GCT TAC ATC CGT TCT CGT 2790
33 I E Q F E A G T G L I L G D A Y I R S R 52

2791 GAT GAA GGT AAA ACC TAC TGT ATG CAG TTC GAG TGG AAA AAC AAA GCA TAC ATG GAC CAC 2950
53 D E G K T Y C M Q F E W K N K A Y M D H 72

2851 GTA TGT CTG CTG TAC GAT CAG TGG GTA CTG TCC CCG CCG CAC AAA AAA GAA CGT GTT AAC 2910
73 V C L L Y D Q W V L S P P B K K E R V N 92

2911 CAC CTG GGT AAC CTG GTA ATC ACC TGG AGC GCC CAG ACT TTC AAA CAC CAA GCT TTC AAC 2970
93 H L G N L V I T W G A Q T F K B Q A F N 112

2971 AAA CTG GCT AAC CTG TTC ATC GTT AAC AAC AAA AAA ACC ATC CCG AAC AAC CTG GTT GAA 3030
113 K L A N L F I V N N K K T I P N N L V E 132

3031 AAC TAC CTG ACC CCG ATG TCT CTG GCA TAC TGG TTC ATG GAT GAT GGT GGT AAA TGG GAT 3090
133 N Y L T P M S L A Y W F M D D G G K W D 152

3091 TAC AAC AAA AAC TCT ACC AAC AAA TCG ATC GTA CTG AAC ACC CAG TCT TTC ACT TTC GAA 3150
153 Y N K N S T N K S I V L N T Q S F T F E 172

3151 GAA GTA GAA TAC CTG GTT AAG GGT CTG CGT AAC AAA TTC CAA CTG AAC TGT TAC GTA AAA 3210
173 E V Z Y L V K G L R N K F Q L N C Y V K 192

3211 ATC AAC AAA AAC AAA CCG ATC ATC TAC ATC GAT TCT ATG TCT TAC CTG ATC TTC TAC AAC 3270
193 I N K N K P I I Y I D S M S Y L I F Y N 212

3271 CTG ATC AAA CCG TAC CTG ATC CCG CAG ATG ATG TAC AAA CTG CCG AAC ACT ATC TCC TCC 3330
213 L I K P Y L I P Q M M Y K L P N T I S S 232

3331 GAA ACT TTC CTG AAA TAA
233 E T F L K *

2. DNA comprising the nucleotide sequence as claimed in claim 1 operatively linked to a promoter.

3. An isolated RNA sequence complementary to the nucleotide sequence of claim 1.

4. RNA complementary to the nucleotide sequence of claim 2.

5. A vehicle comprising a vector containing the nucleotide sequence as claimed in claim 1.

6. The vehicle as claimed in claim 5, wherein the vector is an SV-40 vector.

7. The vehicle as claimed in claim 5, wherein the vector is plasmid pSVOAL.

8. The vehicle as claimed in claim 5 having the identifying characteristics of the vector having culture collection accession number C.N.C.M. I-1014.

9. The vehicle as claimed in claim 5, wherein the vector is an expression vector.

10. A method of genetically mapping a eukaryotic genome that does not contain a natural restriction site for I-SceI, comprising the steps of:

- (a) artificially inserting one or more I-SceI sites at various positions in the genome;
- (b) completely cleaving said genome at the inserted I-SceI sites, with the restriction enzyme I-SceI, to produce nested chromosomal fragments;
- (c) purifying said fragments of step (b) by pulsed field gel electrophoresis (PFG);
- (d) transferring the fragments to a solid membrane;
- (e) hybridizing the fragments bound to said membrane to a labelled probe containing DNA complementary to said fragments;

(f) detecting the hybridization banding patterns; and
(g) mapping said eukaryotic genome based on the hybridization banding patterns observed in step (f).

11. The method of claim 10, wherein said eukaryotic genome is the yeast genome.

12. The method of claim 10, wherein said eukaryotic genome is the genome of the yeast artificial chromosome vector (YAC).

13. The method of claim 10, wherein said step of artificially inserting one or more I-SceI sites comprises random insertion. *D*

14. The method of claim 10, wherein said step of artificially inserting one or more I-SceI sites comprises homologous recombination.

15. The method of claim 11, wherein the probe of step (e) is derived from a cosmid clone, pUKG040.

16. The method of claim 11, wherein the probe of step (e) is derived from a cosmid clone, pUKG066.

17. The method of claim 10, wherein the nested chromosomal fragments of step (c) are used as hybridization probes to sort cosmid libraries.

18. The method of claim 10, wherein after step (b), the genome is partially digested with bacterial restriction enzymes of choice and then electrophoresed, as in step (c), with size calibration markers.

19. A method for *in vivo* site directed genetic recombination in an organism using enzyme I-SceI, comprising the steps of:

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(a) introducing a synthetic gene encoding the I-SceI endonuclease into an expression vector;

(b) inserting a I-SceI restriction site next to or within a gene of interest carried on a plasmid;

(c) co-transforming the cells of said organism with said expression vector of step (a) and said plasmid of step (b), whereby said gene of interest, carried by said plasmid of step (b), is inserted into a chromosome of said organism at a specific site.

20. The method of claim 19, wherein said organism is yeast.

21. The method of claim 19, wherein said organism is bacteria.

22. The method of claim 19, wherein said organism is mouse.

23. The method of claim 19, wherein said synthetic gene of step (a) is under the control of a galactose inducible promoter.

24. The method of claim 23, wherein said expression vector is plasmid pPEX408.

25. The method of claim 23, wherein said expression vector is plasmid pPEX7.

26. A method of genetically mapping a genome that does not contain a natural restriction site for I-SceI, comprising the steps of:

(a) artificially inserting one or more I-SceI sites at various positions in the genome;

(b) completely cleaving said genome at the inserted I-SceI sites, with the restriction enzyme I-SceI, to produce nested chromosomal fragments;

(c) purifying said fragments of step (b); and

(d) mapping said eukaryotic genome by detecting said fragments.

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